

**REMARKS**

The present application teaches a method of regenerating tissues of the multipurpose, fast growing and nitrogen fixing elite tropical legume tree *Acacia mangium*, as well as a method for the genetic transformation of this plant. Claims 14-24 and 28-36, which are directed to methods of transforming *Acacia mangium* with a gene of interest and to a method of preparing transgenic *Acacia mangium* cells, are presently pending. Claims 15-17, 19 and 32-33 have been determined to be free of the prior art.

**Rejections Under 35 U.S.C. §112, Second Paragraph**

Claims 14-24 and 28-36 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for alleging failing to particularly point out and distinctly claim the subject matter which the Applicants regard as their invention. Applicants respectfully transverse this rejection for the reasons set forth below.

The recitations "activating" and "activated" used in claim 14 are asserted to be undefined. Applicant's respectfully disagree. Step A of the method of claim 14 is "activating *Agrobacterium tumefaciens* comprising said gene of interest." It is well known that prior to use in a transformation protocol, *Agrobacterium tumefaciens* must be grown under conditions that promote its ability to transfer the gene of interest contained on the vector plasmid of the *Agrobacterium* to the plant cells that are being transformed. The Examiner's attention is drawn to part A of Example 6 of the present application, entitled "activation of *Agrobacterium tumefaciens* Strain LBA4404/pBI121. Part A of Example 6 provides one illustrative protocol that can be used to activate the *Agrobacterium* to be used in the transformation. Thus, Applicants assert that the first recitation of "activating" or "activated" in claims 14 and all subsequent recitations are understood by those of skill in the relevant art. Moreover, Applicants specifically provide one such protocol for activating *Agrobacterium* in their specification and in claim 34 as presently amended. Applicant's respectfully ask that the rejection under 35

U.S.C. §112, second paragraph be withdrawn as it applies to the use of the terms "activating" and/or "activated" in the pending claims.

The Examiner further asserts that the metes and bounds of the term "preculturing" as used in claim 14 have not been defined. Again, Applicant's respectfully disagree and ask that the rejection be withdrawn. The term "preculturing" is used to indicate the tissue explants such as the stem pieces used in Example 6 are cultured under the appropriate conditions before they are exposed to the activated *Agrobacterium*. A specific example of the preculturing step is given in part B of Example 6. Upon having the disclosure of the present application, those of skill in the art of plant transformation will easily be able use and perhaps modify the protocol for preculturing the explants for use in the claimed transformation procedure. Thus, Applicant's respectfully ask that the rejection under 35 U.S.C. §112, second paragraph be withdrawn as it applies to the use of the term "preculturing" used in the claims.

The Examiner further asserts that the term "AM-265" is not defined. The Examiner specifically cites to the abbreviation TDZ used to designate a component of "AM-265." Applicants respectfully disagree. The components of "AM-265" are well known to those of skill in the art. Moreover, the Examiner is referred to page 4 of the specification at line 4, where it is stated "medium AM-265 (MS basic medium with thidiazuron (1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea or TDZ) . . . ." The Examiner is also referred to the chart at the top of page 8 of the specification, which lists several plant growth regulators used in the present invention, including TDZ, which is also known as thidiazuron. Applicants respectfully ask that the rejection under 35 U.S.C. §112, second paragraph be withdrawn as it applies to the use of the terms "AM-265" and "TDZ" in the pending claims.

With regard to the term "mannitol solution" used in claim 33, the Examiner is again referred to Example 6, part C, which describes soaking stem pieces in a 0.5 M mannitol solution following the preculturing step, just prior to being incubated with the "activated" suspension culture of *Agrobacterium* strain pBI121/LBA4404. Claim 33 has

been amended to indicate the concentration. Likewise the induction medium of claim 34 is the medium used for infecting explants or callus or a cell suspension of *Acacia mangium*. The formula for a representative induction medium is found at page 11 of the application.

It is believed that all terms mentioned by the Examiner are understood by those of skill in the art of plant transformation. Moreover, the specification exemplifies each solution or method step cited by the Examiner. Thus, Applicants believe the rejection under 35 U.S.C. §112, second paragraph, was made in error and it is respectfully asked that the rejection be withdrawn.

#### **Rejections Under 35 U.S.C. §112, First Paragraph**

Claims 14-24 and 28-36 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which is not described in the specification so as to enable one skilled in the art to make and/or use the disclosed inventions. Applicants respectfully ask that this rejection be withdrawn as it appears to have been made in error.

Applicants respectfully assert that the Office may not interpret terms as it sees fit to support a rejection of the claims. Terms in the claims must read as they are understood by those of skill in the art, in view of the teachings of the present specification. The present inventors are the first to develop and disclose a method for transforming and for regenerating tissues of the multipurpose, fast growing and nitrogen fixing elite tropical legume tree *Acacia mangium*. Thus, they are entitled to claims of a scope directed to a method for transforming members of this unique plant species.

The present specification provides more than adequate guidance to those of skill in the relevant art upon which those person can rely to successively carry out the claimed methods. Applicant's statements about the recalcitrance of *Acacia magnum* to tissue culture or transformation are relevant only to establish the state of the art of transformation for this particular species before Applicants provided others with the

teachings they need to carry out what had not been carried out before – the transformation of this particular plant species.

The prior art teaches no conditions and chemical components capable of achieving the results demonstrated in the present application the transformation and regeneration of tissues of *Acacia mangium*. There is no basis for the Examiner to question that the present application does not enable the present invention as broadly as it is presently claimed. The Hansen reference cited by the Examiner supports the Applicant's present position that specific nonobvious alterations of the general procedures used for other specific plant species adapted for use with a unique plant species to provide novel, nonobvious methods for transforming that unique plant species of the present application. In the present case those procedures involve activating the *Agrobacterium* prior to co-cultivation and preculturing the particular *Acacia mangium* explant used in the transformation; co-culturing the explants and *Agrobacterium* to produce infected explants, then culturing infected explants to induce callus and adventitious buds which can then be cultured on selective medium.

The present claims are limited to a method of transforming a single plant species *Acacia mangium*. The teachings of the state of the art indicate that prior to the disclosure of the present inventors, there was no method available for transforming this unique plant species. The present disclosure has removed the unpredictability in the art as to methods for transforming *Acacia mangium*. Applicant's respectfully ask that the rejection under 35 U.S.C. §112, first paragraph, be withdrawn.

#### **Rejections under 35 U.S.C. §103(a)**

Claims 14, 18, 20-22 28-31 and 34-36 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Edwards et al (July 1997, WO 97/23126) in view of Bhaskar et al. (1996, *Indian Journal of Experimental Biology* 34:590-591.) This rejection is apparently based on the Examiner's incorrect assertion that the Office may interpret the term "induction medium" used in the present claims to read on any medium. Edwards

*et al.* teach a method for transformation of a Eucalyptus tree species. Eucalyptus trees are in no way similar to the *Acacia mangium* tress of the present invention. Possession of a method of transforming a Eucalyptus tree provides no predictability that a method of transforming *Acacia mangium* will be successful. See *In re Goodman* 11 F.3d 1046, 1052 (Fed. Cir. 1993) finding that on Goodman's filing date, the record showed no reliable gene transformation method for use with monocot plants. Each method for monocot plants was fraught with unpredictability. Nothing in WO 97/23126 taken alone or together with Bhaskar provides any teaching that overcomes the unpredictability of existing methods for transformation of *Acacia mangium* prior to the filing date of the present application.

Moreover, Bhaskar *et al.* do not teach a method for transforming *Acacia mangium* plants or cells. Nothing in WO 97/23126 or in Bhaskar *et al.*, taken individually or together provides the method of the present invention. Bhaskar reports the results of a study of the response of nodal bud explants of *Acacia mangium* in MS medium supplemented with various concentration of two plant growth regulators.

Recognizing the value of transforming a tree for genetic improvement (WO 97/23126) provides no basis for a reasonable expectation for success in transforming *Acacia mangium*. The preliminary results of Bhaskar *et al.* add nothing to the inadequate disclosures of WO 97/23126. Applicants respectfully assert that the Examiner has failed to make a *prima facie* case of obviousness based on these two references.

Claims 14, 18, 20-22 28-31 and 34-36 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Edwards *et al.* (July 1997, WO 97/23126) in view of Bhaskar *et al.* (1996, *Indian Journal of Experimental Biology* 34:590-591), further in view of Mohamed. Mohamed reports that Murashige and Skoog medium supplemented with 2  $\mu$ M 6-benzylaminopurine (BA) induced adventitious shoots on mature endosperm explants of *Passiflora foetida*, while gibberellic acid and casein hydrolysate stimulated growth and development of shoot primorida. Again, *Passiflora foetida*

is a plant species commonly known as stinking passion flower, passion flower or pop vine, is a plant species totally unrelated to *Acacia mangium*. There is simply no basis for asserting that the results of Mohamed in this totally unrelated plant species add anything to disclosure of the two previously recited references to add to the Examiner's defective Section 103(a) obviousness argument. The citation of Mohamed does not overcome the lack of a *prima facie* case of obviousness discussed above.

Applicants believe that the rejection of claims 14, 18, 20-22 28-31 and 34-36 under 35 U.S.C. § 103(a) is made in error and respectfully ask that the rejection be withdrawn.

Applicants believe the claims in the present application are in condition for allowance and respectfully request a timely notice to that effect. Should additional issues arise that can be effectively dealt with in a timely discussion with Applicant's representative, the Examiner is respectfully asked to contact the same so that the case can be quickly passed to issue.

Respectfully submitted,

By



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